Amendment to Claims

Pursuant to 37 C.F.R. §1.121 the following is a complete listing of the claims of the present application. In this set of claims, please cancel or amend the claims as indicated in the following listing. The following listing of claims will replace all prior versions, and listings, of claims in the application:

1. [Currently amended] An isolated peptide comprising a sequence of at least ~~four~~ six amino acids defined by formula $\underline{P_3}P_2P_1$-P_1'P_2'$\underline{P_3'}$ wherein

P_3 is an uncharged polar amino acid, an uncharged aliphatic amino acid, or an aromatic amino acid,

P_2 is a charged amino acid, a polar amino acid, or an aliphatic amino acid but is not an aromatic amino acid;

P_1 is an aromatic amino acid or an aliphatic amino acid but not a polar amino acid or a charged amino acid;

P_1' is a charged amino acid, or aliphatic amino acid, or a polar amino acid but is not an aromatic amino acid;

P_2' is an uncharged aliphatic polar amino acid or an aromatic amino acid;

wherein P_3' is any amino acid,

and

wherein said peptide is cleaved between P_1 and P_1' by a human aspartyl protease encoded by the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:3 and said peptide does not comprise the corresponding $\underline{P_3}P_2P_1$--P_1'P_2'$\underline{P_3'}$ portion of amino acid sequences depicted in SEQ ID NO:19; SEQ ID NO:20; SEQ ID NO:21; SEQ ID NO:26; SEQ ID NO:27; SEQ ID NO:28; SEQ ID NO:31; SEQ ID NO:32; SEQ ID NO:33; SEQ ID NO:34; SEQ ID NO:35; SEQ ID NO:36; SEQ ID NO:37; SEQ ID NO:38; SEQ ID NO:39; or SEQ ID NO:40.

2. [Cancelled]

3. [Cancelled]

4. [Currently amended] The isolated peptide of claim ~~3~~ 1, comprising an amino acid sequence defined by formula $P_4P_3P_2P_1$--$P_{1'}P_{2'}P_{3'}$, wherein said P_4 is a charged amino acid, a polar amino acid or an aliphatic amino acid but not an aromatic amino acid and said peptide does not comprise the corresponding $P_4P_3P_2P_1$--$P_{1'}P_{2'}P_{3'}$ portion of amino acid sequences depicted in SEQ ID NO:19; SEQ ID NO:20; SEQ ID NO:21; SEQ ID NO:26; SEQ ID NO:27; SEQ ID NO:28; SEQ ID NO:31; SEQ ID NO:32; SEQ ID NO:33; SEQ ID NO:34; SEQ ID NO:35; SEQ ID NO:36; SEQ ID NO:37; SEQ ID NO:38; SEQ ID NO:39; or SEQ ID NO:40.

5. [Currently amended] The isolated peptide of ~~any one of claims 2 through~~ claim 4, further comprising an amino acid at position $P_{4'}$ immediately to the carboxy-terminal position of $P_{3'}$, wherein said $P_{4'}$ is any amino acid said, and wherein the peptide does not comprise the corresponding $P_3P_2P_1$--$P_{1'}P_{2'}P_{3'}P_{4'}$ portion of amino acid sequences depicted in SEQ ID NO:19; SEQ ID NO:20; SEQ ID NO:21; SEQ ID NO:26; SEQ ID NO:27; SEQ ID NO:28; SEQ ID NO:31; SEQ ID NO:32; SEQ ID NO:33; SEQ ID NO:34; SEQ ID NO:35; SEQ ID NO:36; SEQ ID NO:37; SEQ ID NO:38; SEQ ID NO:39; or SEQ ID NO:40.

6. [Currently amended] The isolated peptide of claim ~~1~~ 4, wherein

said P_2 is an amino acid selected from the group consisting of N, L, K, S, G, T, D, A, Q and E;

said P_1 is an amino acid selected from the group consisting of Y, L, M, Nle, F, and H;

said $P_{1'}$ is an amino acid selected from the group consisting of E, A, D, M, Q, S and G;

said $P_{2'}$ is an amino acid selected from the group consisting of V, A, N, T, L, F, and S;

said $P_{3'}$ is an amino acid selected from the group consisting of E, G, F, H, cysteic acid and S;

P_3 is an amino acid selected from the group consisting of A, V, I, S, H, Y, T and F

P_4 is an amino acid selected from the group consisting of E, G, I, D, T, cysteic acid and S; and

$P_{4'}$ is an amino acid selected from the group consisting of F, W, G, A, H, P, G, N, S, and E.

7. [cancelled]

8. [cancelled]

9. [cancelled]

10. [cancelled]

11. [cancelled]

12. [cancelled]

13. [cancelled]

14. [Currently amended] The isolated peptide of ~~any one of claims 1 through 13~~ claim 1 further comprising a first label.

15. [Original] The isolated peptide of claim 14 further comprising a second label.

16. [currently amended] ~~An~~ The isolated peptide ~~according to any one of claims 1-13~~ of claim 1, further comprising a detectable label and a quenching moiety, wherein cleavage of the peptide between P_1 and P_1, separate the quenching moiety from the label to permit detection of the label.

17. [currently amended] The isolated peptide of claim ~~10 or 12~~ 6, wherein said cysteic acid at position P_3, or P_4 further comprises a covalently attached label.

18. [currently amended] The isolated peptide of ~~any one of claims 1-17~~ claim 1, wherein the rate of cleavage of said peptide by said human aspartyl protease is greater than the rate of cleavage of a polypeptide comprising the human APP β-secretase cleavage sequence: SEVKM-DAEFR (SEQ ID NO:20).

19. [currently amended] The isolated peptide of ~~any one of claims 1-17~~ claim 1, wherein the rate of cleavage of said peptide by said human aspartyl protease is greater than the rate of cleavage of a polypeptide comprising the human APP Swedish KM→NL mutation, β-secretase cleavage sequence SEVNL-DAEFR (SEQ ID NO:19).

20. [original] The isolated peptide of claim 1, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; SEQ ID NO:15; SEQ ID NO:16; SEQ ID NO:17, SEQ ID NO:18; SEQ ID NO:120; SEQ ID NO:133; SEQ ID NO:134; SEQ ID NO:135; SEQ ID NO:136; SEQ ID NO:137; SEQ ID NO:138; SEQ ID NO:141; SEQ ID NO:143; SEQ ID NO:144; SEQ ID NO:145; SEQ ID NO:147; SEQ ID NO:148; SEQ ID NO:149; SEQ ID NO:150; SEQ ID NO:151; SEQ ID NO:152; SEQ ID NO:153; SEQ ID NO:154; SEQ ID NO:155; SEQ ID NO:156; SEQ ID NO:157; SEQ ID NO:158; SEQ ID NO:159; SEQ ID NO:160; SEQ ID NO:161; SEQ ID NO:162; SEQ ID NO:163; SEQ ID NO:164; SEQ ID NO:165; SEQ ID NO:166; SEQ ID NO:167; SEQ ID NO:168; SEQ ID NO:169; SEQ ID NO:190; SEQ ID NO:191; SEQ ID NO:192 and SEQ ID NO:193.

21. [currently amended] ~~An~~ The isolated peptide of claim 1 ~~comprising a sequence of at least four amino acids defined by formula $P_3P_2P_1-P_1'P_2'P_3'$,~~ wherein:

P_2 comprises an amino acid selected from the group consisting of N, S, and D;

P_1 comprises an amino acid selected from the group consisting of Y, L, and Nle;

P_1' comprises an amino acid selected from the group consisting of E, A, and D;

P_2' comprises an amino acid selected from the group consisting of A and V; and

wherein a human Aspartyl protease encoded by the nucleic acid sequence of SEQ ID NO: 1 or SEQ ID NO: 3 (Hu-Asp2) cleaves said peptide between P_1 and P_1';

with the proviso that if P_1-P_2- comprise the sequence DA, P_2P_1 do not comprise the sequences NL or NNle.

22. [currently amended] An isolated peptide according to claim 21, wherein the peptide amino acid sequence consists of ~~4-50~~ 6 to 50 amino acids.

23. [original] An isolated peptide according to claim 21, wherein the Hu-Asp2 cleaves the peptide at a rate greater than the Hu-Asp2 cleaves a corresponding peptide having the P_2P_1--P_1-P_2- amino acid sequence KMDA.

24. [currently amended] An isolated peptide according to claim 21, wherein the Hu-Asp2 cleaves the peptide at a rate greater than the Hu-Asp2 cleaves a corresponding peptide having the P_2P_1--P_1-P_2- amino acid sequence ~~KMDA~~ KMNL.

25. [original] A peptide according to claim 21, further comprising a label.

26. [original] A peptide according to claim 21, further comprising a label and a quenching moiety that quenches the label, wherein the label and quenching moiety are attached on opposite sides of the P_1--P_1- peptide bind, whereby cleavage of the P_1--P_1- peptide bond separates the label and quenching moiety.

27. [original] A polypeptide comprising a peptide sequence according to claim 21, and further comprising a transmembrane domain to localize the polypeptide to a cellular membrane when the polypeptide is expressed in a eukaryotic cell.

28. [currently amended] A polypeptide comprising a peptide according to ~~any one of claims 1-20~~ claim 21 and further comprising a transmembrane domain amino acid sequence.

29. [cancelled]

30. [cancelled]

31. [cancelled]

32. [cancelled]

33. [cancelled]

34. [cancelled]

35. [cancelled]

36. [currently amended] A polynucleotide comprising a nucleotide sequence that encodes ~~a polypeptide according to any one of claims 20-35 or~~ a peptide according to claim 1.

37. [cancelled]

38. [original] A vector comprising a polynucleotide according to claim 36.

39. [cancelled]

40. [cancelled]

41. [original] A host cell transformed or transfected with a polynucleotide according to claim 36.

42. [cancelled]

43. [currently amended] A method for assaying for modulators of β- secretase activity, comprising the steps of:

(a) contacting a first composition with a second composition both in the presence and in the absence of a putative modulator compound, wherein the first composition comprises a mammalian β-secretase polypeptide or biologically active fragment thereof, and wherein the second composition comprises a substrate, wherein said substrate comprises a peptide according to ~~any of claims~~ 1 claim 1 ~~through 26~~ or a polypeptide ~~according to any of claims 27-35~~ comprising a peptide of claim 1;

(b) measuring cleavage of the substrate ~~peptide~~ in the presence and in the absence of the putative modulator compound; and

(c) identifying modulators of β- secretase activity from a difference in cleavage in the presence versus in the absence of the putative modulator compound, wherein a modulator that is a β-secretase antagonist reduces such cleavage and a modulator that is a β-secretase agonist increases such cleavage.

44. [cancelled]

45. [cancelled]

46. [cancelled]

47. [cancelled]

48. [cancelled]

49. [currently amended] The method claim of ~~any of claims 43-48~~ claim 43, further comprising a step of treating Alzheimer 's Disease with an agent identified as an inhibitor of Hu-Asp2.

50. [cancelled]

51. [cancelled]

52. [original] A method of producing a substrate for a β-secretase assay comprising:

growing a host cell transformed or transfected with a vector of claim 40 in a manner allowing expression of said polypeptide.

53. [cancelled]

54. [cancelled]

55. [cancelled]

56. [cancelled]

57. [cancelled]

58. [currently amended] A method for identifying agents that inhibit the activity of human Asp2 aspartyl protease (Hu-Asp2), comprising the steps of:

(a) contacting a peptide of ~~any of claims 1 through 26~~ claim 1 or a polypeptide ~~of any of claims 27-35~~ comprising a peptide of claim 1 and a composition comprising an Hu-Asp2 activity in the presence and absence of a test agent;

(b) determining the cleavage of said peptide or polypeptide between said P1 and P1' by said Hu-Asp2 in the presence and absence of the test agent; and

(c) comparing said cleavage activity of the Hu-Asp2 in the presence of the test agent to the activity in the absence of the test agent to identify an agent that inhibits said cleavage by the Hu-Asp2, wherein reduced activity in the presence of the test agent identifies an agent that inhibits Hu-Asp2 activity.

59. [original] A method according to claim 58, wherein the Hu-Asp2 is a recombinant Hu-Asp2 purified and isolated from a cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes Hu-Asp2.

60. [original] A method according to claim 58,

wherein the Hu-Asp2 is expressed in a cell, wherein the contacting comprises growing the cell in the presence and absence of the test agent, and

wherein the determining step comprises measuring cleavage of said peptide or fusion protein.

61. [cancelled]

62. [cancelled]

63. [currently amended] A method according to ~~any one of claims~~ claim 59-~~62~~ wherein the nucleotide sequence is selected from the group consisting of:

(a) a nucleotide sequence encoding the Hu-Asp2(a) amino acid sequence set forth in SEQ ID NO: 2;

(b) a nucleotide sequence encoding the Hu-Asp2(b) amino acid sequence set forth in SEQ ID NO: 4;

(c) a nucleotide sequence encoding a fragment of Hu-Asp2(a) (SEQ ID NO: 2) or Hu-Asp2(b) (SEQ ID NO: 4), wherein said fragment exhibits aspartyl protease activity characteristic of Hu-Asp2(a) or Hu-Asp2(b); and

(d) a nucleotide sequence of a polynucleotide that hybridizes under stringent hybridization conditions to a Hu-Asp2-encoding polynucleotide selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 3.

64. [currently amended] A method for identifying agents that modulate the activity of Asp2 aspartyl protease, comprising the steps of:

(a) contacting an Asp2 aspartyl protease and a peptide ~~of any of claims 1 through 26~~ of claim 1 or a polypeptide of ~~any of claims 27 through 35~~ comprising a peptide of claim 1 in the presence and absence of a test agent, wherein the Asp2 aspartyl protease is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a Hu-Asp2-encoding polynucleotide selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 3;

(b) determining the cleavage of said peptide or fusion protein between said P_1 and said $P_{1'}$ site by said Asp2 in the presence and absence of the test agent; and

(c) comparing the cleavage activity of said Asp2 in the presence of the test agent to the cleavage activity in the absence of the agent to identify agents that modulate the activity of the polypeptide, wherein a modulator that is an Asp2 inhibitor reduces said cleavage and a modulator that is an Asp2 agonist increases said cleavage.

65. [cancelled]

66. [original] A method for identifying agents that inhibit the activity of human Asp2 aspartyl protease (Hu-Asp2), comprising the steps of:

(a) growing a cell in the presence and absence of a test agent, wherein the cell expresses an Hu-Asp2 and expresses a protein comprising a peptide of ~~any of claims 1 through 26~~ claim 1 or a polypeptide of ~~any of claims 27 through 35~~ comprising a peptide of claim 1;

(b) ~~determining the~~ determining the cleavage of said protein at the site between said P1 and P1' in said cell in the presence and absence of the test agent; and

(c) comparing said cleavage activity in the presence of the test agent to the cleavage activity in the absence of the test agent to identify an agent that inhibits the activity of Hu-Asp2, wherein reduced cleavage activity in the presence of the test agent identifies an agent that inhibits Hu-Asp2 activity.

67. [cancelled]

68. [cancelled]

69. [cancelled]

70. [currently amended] A kit for performing a β-secretase assay comprising a β-secretase substrate comprising a peptide according to ~~any of claims 1 through 27~~ claim 1 and a β-secretase enzyme.

71. [cancelled]

72. [currently amended] The kit of claim 70 ~~or 71~~, further comprising reagents for detecting the cleavage of said peptide or fusion protein.

73. [original] An isolated peptide comprising a sequence of at least 10 amino acids having the sequence SEISY-EVEFR (SEQ ID NO:152).

74. [original] The isolated peptide of claim 73, wherein said peptide comprises at least 3 amino acids immediately to the carboxy-terminal of SEISY-EVEFR (SEQ ID NO:152).

75. [original] The isolated peptide of claim 73, wherein said peptide comprises at least 3 amino acids immediately to the amino-terminal of SEISY-EVEFR (SEQ ID NO:152).

76. [original] The isolated peptide of claim 73, wherein said peptide comprises at least 5 amino immediately to the carboxy-terminal of SEISY-EVEFR (SEQ ID NO:152).

77. [original] The isolated peptide of claim 73, wherein said peptide comprises at least 5 amino immediately to the amino-terminal of SEISY-EVEFR (SEQ ID NO:152).

78. [original] The isolated peptide of claim 73, wherein said peptide comprises at least 10 amino immediately to the amino-terminal of SEISY-EVEFR (SEQ ID NO:152).

79. [original] The isolated peptide of claim 73, wherein said peptide comprises at least 13 amino acids.

80. [original] The isolated peptide of claim 73, wherein said peptide comprises at least 15 amino acids.

81. [original] The isolated peptide of claim 73, wherein said peptide comprises at least 20 amino acids.

82. [original] The isolated peptide of claim 73, wherein said peptide comprises at least 50 amino acids.

83. [NEW] The isolated peptide of claim 21, wherein said peptide comprises a sequence of P_3P_2Y-EVE, P_3P_2Y-AVE, P_3P_2Y-DVE, or P_3P_2Y-DAE and a human aspartyl protease encoded by the nucleic acid sequence of SEQ ID NO: 1 or SEQ ID NO: 3 (Hu-Asp2) cleaves said peptide at the bond between Y and E, and P_3P_2 is selected from the group consisting of IS, ID, IN, VS, VD, and VN.